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Specification and Drawings, as originally filed, with Application for Patent Serial No:  
**2,423,327**, on April 2, 2003, by **PHARMACOGENETICS LIMITED**, assignee of Hong  
Xue and Lo Wing Sze, for "Single Nucleotide Polymorphisms for the Diagnosis and  
Treatment of Schizophrenia Spectrum Disorders".

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(CIPO 68)  
04-09-02

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...prototype as, or  
diagnostic and drug development



1 ABSTRACT

2  
3 Six single nucleotide polymorphisms (SNPs) are found to be associated with  
4 schizophrenia. The applications of these SNPs, and genetic markers in the same haplotype as, or  
5 linkage disequilibrium with, one or more of these six SNPs to diagnostic and drug development  
6 purposes are described in the present invention.

**SINGLE NUCLEOTIDE POLYMORPHISMS  
FOR THE DIAGNOSIS AND TREATMENT  
OF SCHIZOPHRENIA SPECTRUM DISORDERS**

**BACKGROUND OF THE INVENTION**

**FIELD OF THE INVENTION**

**[0001]** This invention relates to the diagnosis and treatment for psychotic and affective disorders, such as schizophrenia. This invention also relates to methods of screening for therapeutics for schizophrenia and other psychotic and affective disorders.

**DESCRIPTION OF THE PRIOR ART**

**[0002]** Common human neuropsychiatric disorders including psychotic and affective disorders are multi-factorial or complex in nature. The family of disorders that share clinical and often etiologic (i.e., genetic) characteristics with schizophrenia have been referred to as the "schizophrenia spectrum", which covers schizophrenia, nonschizophrenic, nonaffective psychotic disorders (i.e., schizophreniform disorder, delusional disorder, schizoaffective disorder, atypical psychosis), schizophrenia-like personality disorders and some affective disorders (A. Breier, 1999. Diagnostic classification of the psychoses: historical context and implications for neurobiology. In: Neurobiology of Mental Illness. D. S. Charney, E. J. Nestler, B. S. Bunney (Ed.) Oxford University Press. Chapter 15. pp. 197 – 198). To date, linkage studies have been relatively unsuccessful in identifying genes associated with complex disorders such as bipolar disorder, schizophrenia, depression, anxiety-related traits and autism; few loci have yet been unequivocally identified.

**[0003]** Schizophrenia is one of the most common and serious psychiatric disorders and is characterised by a profound disruption of cognition and emotion, affecting fundamental human attributes such as language, thought, perception, and sense of self. The disease is accompanied by an array of symptoms which frequently include hallucination and delusion. Studies of the prevalence of schizophrenia in the general population demonstrated that, in general, 1-year

prevalence in adults between the ages of 18 to 54 is estimated to be 1.3 percent. Onset generally occurs during young adulthood (mid-20s for men, late-20s for women), although earlier and later onsets do occur. Onset of the disease may be abrupt or gradual, but most people experience some early signs, such as increasing social withdrawal, loss of interest, unusual behavior, or decreases in functioning prior to the beginning of active positive symptoms.

[0004] At this time, a clear consensus as to the etiopathogenesis of schizophrenia has yet to be established. The most popularly held opinion points to the interaction of genetic factors and major environmental upheaval during development of the brain. Family, twin, and adoption studies support the role of genetic influences in schizophrenia (Kendler KS and Diehl SR, 1993. The genetics of schizophrenia: a current, genetic-epidemiologic perspective. *Schizophr Bull* 19:261-85; McGuffin P, Owen MJ and Farmer AE, 1995. Genetic basis of schizophrenia. *Lancet* 346: 678-82; Portin P and Alanen YO, 1997. A critical review of genetic studies of schizophrenia. II. Molecular genetic studies. *Acta Psychiatr Scand* 95:73-80). Immediate biological relatives of people with schizophrenia have about a 10-time greater risk than that of the general population. Given prevalence estimates, this translates into a 5 to 10 percent lifetime risk for first-degree relatives (including children and siblings) and suggests a substantial genetic component to schizophrenia (Kety SS, 1987. The significance of genetic factors in the etiology of schizophrenia: results from the national study of adoptees in Denmark. *J Psychiatr Res* 21:423-9; Tsuang MT, 1991. Morbidity risks of schizophrenia and affective disorders among first-degree relatives of patients with schizoaffective disorders. *Br J Psychiatry* 158:165-70). What also bolsters a genetic role are findings that the identical twin of a person with schizophrenia is at greater risk than a sibling or fraternal twin, and that adoptive relatives do not share the increased risk of biological relatives. However, in about 40 percent of identical twins in which one is diagnosed with schizophrenia, the other never meets the diagnostic criteria. The discordance among identical twins clearly indicates that environmental factors likely also play a role. However, despite the evidence for genetic vulnerability to schizophrenia, scientists are only beginning to identify the genes responsible (Kendler KS & Diehl SR, 1993. The genetics of schizophrenia: a current, genetic-epidemiologic perspective. *Schizophr Bull* 19:261-85; Shifman S, Bronstein M, Sternfeld M *et al.*, 2002. A highly significant association between a COMT

1 haplotype and schizophrenia. *Am J Hum Genet* 71: 1296-1302). The current consensus is that  
 2 multiple genes are responsible (Kendler KS, MacLean CJ, O'Neill FA *et al.*, 1996. Evidence for  
 3 a schizophrenia vulnerability locus on chromosome 8p in the Irish Study of High-Density  
 4 Schizophrenia Families. *Am J Psychiatry* 153:1534-40; Kunugi H, Curtis D, Vallada HP *et al.*,  
 5 1996. A linkage study of schizophrenia with DNA markers from chromosome 8p21-p22 in 25  
 6 multiplex families. *Schizophr Res* 22:61-8; Portin P and Alanen YO, 1997. A critical review of  
 7 genetic studies of schizophrenia. II. Molecular genetic studies. *Acta Psychiatry Scand* 95:73-80;  
 8 Straub RE, MacLean CJ, Martin RB, Ma Y, *et al.*, 1998. A schizophrenia locus may be located  
 9 in region 10p15-p11. *Am J Med Genet* 81:296-301).

10 [0005] Excessive levels of the neurotransmitter dopamine have long been implicated in  
 11 schizophrenia, although it is unclear whether the excess is a primary cause of schizophrenia or  
 12 the result of a more fundamental dysfunction. Recent evidence implicates much greater  
 13 complexity in the dysregulation of dopamine and other neurotransmitter systems (Grace AA,  
 14 1991. Phasic versus tonic dopamine release and the modulation of dopamine system  
 15 responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 41:1-24; Grace AA,  
 16 1992. The depolarization block hypothesis of neuroleptic action: implications for the etiology  
 17 and treatment of schizophrenia. *J Neural Transm Suppl* 36:91-131; Olie JP and Bayle FJ, 1997.  
 18 New chemotherapy approaches to psychoses. *Encephal Spec* 2: 2-9). Some of this research ties  
 19 schizophrenia to certain variations in dopamine receptors (Nakamura K, 1995. Symptomologic  
 20 characteristics and psychopathology of expression of an "attack of altered perception" in  
 21 schizophrenic patients. *Seishin Shinkeigaku Zasshi* 97:529-50; Serretti A, Macciardi F &  
 22 Smeraldi E, 1998. Dopamine receptor D2 Ser/Cys311 variant associated with disorganized  
 23 symptomatology of schizophrenia. *Schizophr Res* 34:207-10), while other research focuses on  
 24 the serotonin system (Inayama Y, Yoneda H, Sakai T, *et al.*, 1996. Positive association between  
 25 a DNA sequence variant in the serotonin 2A receptor gene and schizophrenia. *Am J Med Genet*  
 26 67:103-5).

27 [0006] Serotonin is a key neurotransmitter in the central nervous system, and dysregulation  
 28 of serotonergic pathways has been implicated in the pathogenesis of many complex psychiatric  
 29 diseases. Polymorphisms of many of the genes involved in serotonin biosynthesis, catabolism,

and response have been reported, suggesting that genetic variability may underlie the development of diseases such as schizophrenia, obsessive compulsive disorder, and suicide.

[0007] GABA ( $\gamma$ -aminobutyric acid) is a major neurotransmitter that mediates inhibitory transmission. GABA receptors are divided into two types, GABA<sub>A</sub> receptors and GABA<sub>B</sub> receptors. GABA<sub>A</sub> receptors are the major inhibitory neurotransmitter receptors. They are ligand-gated chloride ion channels that possess binding sites for many important drugs thought to act, in part, through modulation of receptor function (Burt DR and Kamatchi GL, 1991. GABA<sub>A</sub> receptor subtypes: from pharmacology to molecular biology. *FASEB* 5: 2916-2923).

[0008] Abnormalities of the GABA system have been implicated in the pathophysiology of schizophrenia (Adel A *et al.*, 1999. Critical review of GABAergic drugs in the treatment of schizophrenia. *J Clin Psychopharmacol* 19: 222-232). Several studies have also shown that in cortical GABA<sub>A</sub> receptors, tritiated muscimol binding and levels of  $\alpha$ ,  $\beta_2$  and  $\beta_3$  subunits of the receptor increased. Moreover, the expression of the short isoform of the  $\gamma_2$  subunit of this receptor may be markedly reduced in the dorsolateral prefrontal cortex (PFC) of schizophrenic subjects (Lewis DA, 2000. GABAergic local circuit neurons & prefrontal cortical dysfunction in schizophrenia. *Brain Research Reviews* 31: 270-276).

[0009] GABA<sub>A</sub> receptors contain at least 18 subunits. They are located on the human chromosomes 1, 4, 5, 6, 15 and X. The  $\alpha$ ,  $\beta_{2,3}$  and  $\gamma$  subunit genes of GABA<sub>A</sub> receptor are genes that could be related to schizophrenia (Adel A *et al.*, 1999. Critical review of GABAergic drugs in the treatment of schizophrenia. *J Clin Psychopharm* 19: 222-232; Lewis DA, 2000. GABAergic local circuit neurons & prefrontal cortical dysfunction in schizophrenia. *Brain Research Reviews* 31: 270-276; Rapadimitrion G *et al.*, 2001. Association between GABA<sub>A</sub> receptor  $\alpha_5$  subunit gene locus and schizophrenia of a later age of onset. *Neuropsychobiology* 43: 141-144)

[0010] The  $\alpha_1$  subunit is present predominantly in the cerebellum whereas the  $\alpha_5$  subunit is prevalent in the hippocampus, and  $\alpha_6$  subunit is expressed almost exclusively within the cerebellar granule cell. The  $\alpha_4$  subunit constitutes a more minor receptor subtype is expressed primarily in the thalamus and the hippocampus (Mehta AK, Maharaj KT, 1999. An update on GABA<sub>A</sub> receptors. *Brain Res Rev* 29: 196-217).

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1 [0011] The  $\beta$  subunit is a key structural and functional component of GABA<sub>A</sub> receptors. It  
 2 was recognized as a potential binding site for GABA (Krishek BJ *et al.*, 1996. Homomeric  $\beta_1$   $\gamma$ -  
 3 aminobutyric acid<sub>A</sub> receptor-ion channels: evaluation of pharmacological and physiological  
 4 properties. *Molecular Pharmacology* 49: 494-504). The importance of  $\beta$  subunit isoforms in  
 5 governing GABA<sub>A</sub> receptor  $\alpha_1$  subunit expression has been suggested (Kumar M *et al.*, 2001.  
 6 Antisense suppression of GABA<sub>A</sub> receptor  $\beta$ s subunit levels in cultured cerebellar granule  
 7 neurons demonstrates their importance in receptor expression. *J Neurochem* 77: 211-219). The  
 8 most prevalent  $\beta$  subunit is  $\beta_2$ , which was most often associated with the  $\alpha_1$  subunit (Stephenson  
 9 FA, 1995. The GABA<sub>A</sub> receptors. *Biochem J* 310: 1-9). The  $\beta_2$  subunit has two isoforms: long  
 10 form  $\beta_{2L}$  and short form  $\beta_{2s}$ . The  $h\beta_{2L}$  cDNA sequence is identical to  $h\beta_{2s}$  except for a 114 base  
 11 pair (38 amino acids) insertion within the large intracellular loop between transmembrane  
 12 regions III and IV. This 114 base pair insertion sequence was searched for consensus  
 13 phosphorylation sites and was found to contain a motif for a calmodulin-dependent protein  
 14 kinase II (CDPK-II) with threonine as the acceptor. It has been proposed that benzodiazepines  
 15 produce their anticonvulsant effects by inhibiting brain calcium or calmodulin-dependent protein  
 16 kinase. Therefore, the CDPK-II motif contained in the  $h\beta_{2L}$  insert could play a role in  
 17 determining the pharmacology of GABA<sub>A</sub> receptors containing  $h\beta_{2s}$  versus  $h\beta_{2L}$  (McKinley DD  
 18 *et al.*, 1995. Cloning, sequence analysis and expression of two forms of mRNA coding for the  
 19 human  $\beta_2$  subunit of the GABA<sub>A</sub> receptor. *Mol Brain Res* 28: 175-179). Changes in  $\beta$  subunit  
 20 composition may alter receptor function and pharmacology in vivo. Modulation of receptor  
 21 function by phosphorylation is also influenced by  $\beta$  subunit composition and contributes to  
 22 increased inhibition during neuronal excitation (Russek SJ *et al.*, 2000. An initiator element  
 23 mediates autologous down-regulation of the human type A  $\gamma$ -aminobutyric acid receptor  $\beta_1$   
 24 subunit gene. *Proc Natl Acad Sci* 97: 8600-8605).

25 [0012] The  $\beta_2$  subunits are most likely associated with the  $\alpha_1$  subunit (Stephenson FA, 1995.  
 26 The GABA<sub>A</sub> receptors. *Biochem J* 310: 1-9). The whole  $\beta_2$  sequence (*GABRB2*) could be  
 27 obtained by re-organising source sequences from the National Center for Biotechnology  
 28 Information (NCBI) database by BLASTing the unorganised whole gene sequence with long  
 29 form (NM\_021911) and short form (NM\_000813) mRNA. Reorganisation of the BLAST result



1 yields the complete sequence and distribution of exons and introns of the  $\beta_2$  subunit gene. The  
2 complete sequence of  $\beta_2$  subunit gene covers about 254 kb. The long form transcript variant  
3 contains 10 exons totalling 1.5 kb, and the short form transcript variant contains 9 exons totalling  
4 1.4 kb. Exon 9 which contains 113 bases in sequence is deleted from the short form transcript.

5 [0013] While a number of reports have suggested a correlation between GABA<sub>A</sub> receptors  
6 and schizophrenia (see above), the exact genetic basis for the correlation remains unclear. It is  
7 the object of the present invention to provide further genetic tools and methods to assist the  
8 diagnosis and treatment of schizophrenia.

9 [0014] Single Nucleotide Polymorphisms

10 [0015] Single nucleotide polymorphisms (SNPs) represent one of the most common forms of  
11 genetic variation. These polymorphisms occur when a single nucleotide (A, G, C or T) in the  
12 genome is altered. The most common form of SNP is the replacement of cytosine with thymine.  
13 SNPs generally tend to be evolutionarily stable from generation to generation and, as such, can  
14 be used to study specific genetic abnormalities throughout a population. SNPs often occur in  
15 protein coding regions and, as a result, may lead to the expression of a defective or variant form  
16 of a protein. Such polymorphisms can therefore serve as effective indicators of genetic disease.  
17 However, not all SNPs are found in protein coding regions of the genome. Some SNPs are  
18 located in noncoding regions, but these polymorphisms may also lead to altered protein  
19 expression. Specifically, SNP sites in noncoding regions may, for example, lead to differential  
20 and defective splicing. In diseases such as schizophrenia, where a large number of genes may  
21 influence the onset of the disease, SNPs can be used as diagnostic tools for identifying  
22 individuals with a predisposition for manifesting the disease, genotyping the patients suffering  
23 from the disease in terms of the genetic causes underlying the condition, and facilitating drug  
24 development based on the insight revealed regarding the role of target proteins in the  
25 pathogenesis process.

26 [0016] When SNPs are identified and genotyped in patients or in families, data must be  
27 analyzed to determine whether the association between an SNP and a disease is significant. Just  
28 as linkage is a relationship between loci, association is a relationship between alleles. Thus, an  
29 alternative to linkage mapping in families is to search for statistical associations between one

1 allele and the disease. The idea behind this approach is that if one allele m1 can directly cause  
2 susceptibility to the disease, generally, possession of m1 is generally not necessary or sufficient  
3 for someone to develop the disease, but its frequency should increase in the proband population  
4 compared to unaffected controls. The same should also be true for all alleles in linkage  
5 disequilibrium (LD) with allele m1. Thus, SNP-based association studies can be performed in  
6 two ways: direct testing of a SNP with functional consequence for association, or using a SNP as  
7 a marker for LD. Therefore, a common core haplotype involving several SNPs should be more  
8 frequent in probands compared to controls.

9 [0017] Five SNPs have been previously reported in the human *GABRB2* gene  
10 (<http://www.ncbi.nlm.nih.gov/SNP/>). These SNPs have been identified as rs1816071,  
11 rs1816072, rs194072, rs252944 and rs187269.

12 [0018] The present invention seeks to provide a set of SNPs and their haplotypes for  
13 diagnoses of psychotic and affective disorders such as schizophrenia and identifying appropriate  
14 target proteins for drug development.

15

#### 16 SUMMARY OF THE INVENTION

17 [0019] In one embodiment, the present invention provides an isolated polynucleotide having  
18 a nucleotide sequence comprising corresponding to SEQ ID NO: 1, the polynucleotide  
19 comprising a portion of the human *GABRB2* gene including a polymorphic site at position 1584  
20 of intron 7.

21 [0020] In another embodiment, the invention provides a PCR primer set for amplifying  
22 regions of a polynucleotide corresponding to SEQ ID NO: 1, the primer set comprising a first  
23 primer having a sequence corresponding or complementary to a sequence corresponding to SEQ  
24 ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 or SEQ ID NO: 12,  
25 and a second primer having a sequence corresponding or complementary to a sequence  
26 corresponding to SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO:  
27 11 or SEQ ID NO: 13.

28 [0021] In yet another embodiment, the invention provides a method of predicting whether an  
29 individual is more or less likely to suffer from schizophrenia comprising:

a) determining the nucleotide sequence from a nucleic acid sample from the individual;  
and

b) identifying DNA sequences corresponding to one or more SNPs selected from the group consisting of: I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 of the human *GABRB2* gene, or other genetic markers in the same haplotype as, or in linkage disequilibrium with, one or more of the SNPs.

[0022] In yet another embodiment, the present invention provides a method of genotyping the schizophrenia-spectrum disorder of a patient in order to provide pharmacogenetic guidance for drug therapy for the patient, comprising:

[0023] a) determining the nucleotide sequence from a nucleic acid sample from a schizophrenic patient; and

[0024] b) identifying DNA sequences corresponding to one or more SNPs selected from the group consisting of: I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 of the human *GABRB2* gene, or other genetic markers in the same haplotype as, or in linkage disequilibrium with, one or more of the the SNPs.

[0025] In another embodiment, the invention provides a method of drug screening for identifying drugs for treating schizophrenic disorders, the method comprising:

a) transfecting a vector into a eukaryotic expression system, the vector containing a DNA sequence corresponding to at least a portion of the human *GABRB2* gene, the portion of the *GABRB2* gene including one or more SNPs chosen from the group consisting of: I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269;

b) expressing the vector in a cellular expression system;

c) adding a drug to be screened into the cellular system;

d) analyzing the expression of the *GABRB2* gene encoding GABA<sub>A</sub> receptor  $\beta_2$  subunit and also analysing the expression and resultant activity of the GABA<sub>A</sub> receptor in the cellular system;

e) determining the effect of the drugs on the expression of the GABA<sub>A</sub>  $\beta_2$  subunit and the GABA<sub>A</sub> receptor, and the activity of the expressed GABA<sub>A</sub> receptor in the cellular system; and,

f) identifying those drugs that alter the levels of the expression of *GABRB2* gene.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0026] These and other features of the preferred embodiments of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

[0027] Figure 1a is a nucleotide sequence of Exon 7, Exon 8 and the Intron 7 between such exons of the human *GABRB2* gene comprising the three SNPs I7G1584T, rs1816071 and rs1816072.

[0028] Figure 1b is a nucleotide sequence of Exon 7, Exon 8 and the Intron 7 between such exons of the human *GABRB2* gene comprising the two SNPs rs194072 and rs252944.

[0029] Figure 2 is a nucleotide sequence of Exon 8, Exon 9 and the Intron 8 between such exons of the human *GABRB2* gene comprising the SNP rs187269.

[0030] Figure 3 shows the locations of the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 of the human *GABRB2* gene.

[0031] Figure 4 shows data obtained from the sequencing gel of the flanking regions around the SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269.

### DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0032] The below terms are used throughout the present application and will be assumed to have the following definitions:

[0033] The term "allele" is used herein to refer to variants of a nucleotide sequence.

[0034] The term "antisense" is used herein, to refer to a complementary strand of a coding sequence of DNA or RNA. Such coding sequence may contain, for example, a SNP of interest. Copies of antisense polynucleotides can be introduced into a cell or organism to inhibit expression of a corresponding gene or to alter the splicing of mRNA in specific regions.

Antisense polynucleotides are entirely or substantially complementary to a target polynucleotide and have the ability to specifically hybridize to such target polynucleotide.

[0034] The terms "complementary" and "complement", as used herein, refer to polynucleotide sequences which are capable of base pairing with another polynucleotide throughout the complementary region.

[0035] The term "haplotype" refers to a series of known DNA sequences linked on a chromosome. The known DNA sequences can be in the form of SNPs or other markers. A haplotype may refer to a combination of polymorphisms found in an individual that may be associated with a phenotype.

[0036] The term "genotype" refers to the genetic constitution of an organism. More specifically, the term refers to the identity of alleles present in an individual. "Genotyping" of an individual or a DNA sample refers to identifying the nature, in terms of nucleotide bases, of a specific allele possessed by an individual at a known polymorphic site.

[0037] The term "polymorphism", as used herein, refers to the coexistence of more than one form of gene or portion thereof in a population. "Polymorphic" refers to the condition in which two or more variants of a specific genomic sequence can be found in a population. A "polymorphic site" refers to a locus at which variations in nucleotide sequence occur.

[0038] The terms "oligonucleotide" and "polynucleotide" as used in the present application refer to RNA, DNA or hybrid RNA/DNA sequences being of greater than one nucleotide in length. Such sequences may exist in either single or double-stranded form.

[0039] The term "PCR", as used herein, refers to the polymerase chain reaction. PCR is a method of amplifying a DNA base sequence using a heat stable polymerase and a pair of primers, one primer complementary to the (+)-strand at one end of the sequence to be amplified and the other primer complementary to the (-) strand at the other end of the sequence to be amplified. Newly synthesized DNA strands can subsequently serve as templates for the same primer sequences and successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. PCR can be used to detect the existence of a defined sequence in a DNA sample.

[0040] The term "primer", as used herein, refers to a short single-stranded oligonucleotide capable of hybridizing to a complementary sequence in a DNA sample. The primer serves as an initiation point for template dependent DNA synthesis. Deoxyribonucleotides can be added to a

1 primer by a DNA polymerase. A "primer pair" or "primer set" refers to a set of primers  
2 including a 5'upstream primer that hybridizes with the 5' end of the DNA sequence to be  
3 amplified and a 3', downstream primer that hybridizes with the complement of the 3' end of the  
4 DNA sequence to be amplified.

5 [0041] The term "exon" refers to the protein coding DNA sequence of a gene.

6 [0042] The term "intron" or "intronic region" refers to DNA base sequences interrupting the  
7 protein-coding sequences of a gene. These sequences are transcribed into RNA but are cut out of  
8 the RNA before it is translated into protein.

9 [0043] The present invention relates, generally, to single nucleotide polymorphisms (SNPs)  
0 associated with the etiopathogenesis of schizophrenia. As found by the present inventors,  
1 individuals possessing certain SNPs, or their complementary regions, have been found to have an  
2 increased susceptibility to a schizophrenia spectrum disorder. These disorders have been linked  
3 to mutations of the *GABRB2* gene. As indicated above, the *GABRB2* gene encodes the  $\beta_2$  subunit  
4 of human gamma-aminobutyric acid type A receptor.

5 [0044] In one aspect, the present invention provides an association of certain SNP sites with  
6 schizophrenia disorders. These SNPs are as follows: rs1816071, rs1816072, rs194072,  
7 rs252944, and rs187269. These SNPs are present in the introns preceding and following Exon 8  
8 of *GABRB2*, and the haplotype or haplotypes containing one or more of these six SNPs. The  
9 above listed SNPs are known in the art but have not been previously associated with  
0 schizophrenia disorders.

1 [0045] According to another embodiment, the present invention provides a further,  
2 previously unknown SNP, also associated with schizophrenia disorders. This SNP, referred to  
3 herein as SNP I7G1584T, is found in intron 7 of the *GABRB2* gene. This intron is the region  
4 between Exons 7 and 8 of the aforementioned gene. The methodology used to identify this SNP  
5 is provided below.

6 [0046] Results of linkage disequilibrium or association analysis of the above mentioned six  
7 SNPs are shown in Tables 1 and 2(provided herein below), which present data concerning the  
8 correlation between each allele and genotype frequency with schizophrenia. In Tables 1 and 2,  
9 "SCH" represents schizophrenic patients and "CON" represents normal controls. Statistical

1 testing of the data shows that the six SNPs I7G1584T, rs1816071, rs1816072, rs194072,  
 2 rs252944 and rs187269 in the  $\beta_2$  subunit gene are significantly associated at the allelic level with  
 3 schizophrenia, with  $p < 0.05$ ; and that the five SNPs I7G1584T, rs1816071, rs194072, rs252944  
 4 and rs187269 are significantly associated at the genotypic level with schizophrenia, with  $p < 0.05$ .  
 5 Therefore, these various SNPs may serve as potential genetic factors linked to the  
 6 etiopathogenesis of schizophrenia.

7 [0047] Accordingly, in one aspect, the present invention provides a method of detecting the  
 3 SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944, and rs187269, and their associated  
 4 haplotypes, for the diagnosis of susceptibility to schizophrenia, the genotyping of cases of  
 5 schizophrenia, and to render GABA<sub>A</sub> receptors containing a  $\beta_2$  subunit as potentially useful  
 6 target protein systems for the screening and development of drugs to treat the disease.

7 [0048] Figure 1a depicts a nucleotide sequence containing Exon 7, Exon 8 and the Intron 7  
 3 between such exons in the human *GABRB2* gene. In Figure 1a, the SNP I7G1584T is identified  
 4 by a rectangular box with solid lines. The SNP rs1816071 is identified by a rectangular box with  
 5 dashed lines, and the SNP rs1816072 is identified by a rectangular box with a double solid line.  
 6 Exon 7 is underlined with a double dashed line. Exon 8 is underlined with a single dashed line.  
 The PCR-amplified region containing I7G1584T, rs1816071 and rs1816072 is underlined with a  
 solid line.

[0049] In Figure 1b, the SNP rs194072 is identified by a rectangular box with solid lines.  
 The SNP rs252944 is identified by a rectangular box with dashed lines. Exon 7 is underlined  
 with a double dashed line. Exon 8 is underlined with a single dashed line. The PCR-amplified  
 region containing rs194072 is underlined with a solid line. The PCR-amplified region containing  
 rs252944 is shown in bold type.

[0050] Referring now to Figure 2, a nucleotide sequence of Exon 8, Exon 9 and the Intron 8  
 between such exons of the human *GABRB2* gene is presented. The SNP rs187269 is identified  
 by a rectangular box with a solid line. Exon 8 is shown in bold type and Exon 9 is underlined  
 with a dashed line. The PCR-amplified region is underlined with a solid line.

[0051] The above-mentioned figures present the SNPs which have been associated with the  
 schizophrenia.

[0052] It is another aspect of the present invention to provide a method of screening and diagnosing the occurrence and genotype of schizophrenia by analyzing the sequences of the GABA receptor  $\beta_2$  gene, namely *GABRB2*, in order to identify the allele and/or genotype and/or haplotype of the subject at SNP I7G1584T, rs1816071, rs1816072, rs194072 or rs252944 or rs187269 or a combination including any number of these six SNPs. The method includes obtaining human DNA in sufficient quantity for sequence analysis, and identifying the nature of the base at one or more of these SNP positions in the human *GABRB2* gene. The method used may involve common techniques such as PCR, under the same conditions and using the same primers as described below in the examples provided for detection of nucleotide sequences associated with schizophrenia, followed by DNA sequence analysis. Alternate primers upstream and downstream of these six SNP positions may also be used and may be determined by one skilled in the art without undue experimentation. Any other methods useful for SNP genotype and haplotype analysis may be applied to identify the variants at the six SNP sites and other markers in the same haplotype as, or in linkage disequilibrium with, the six SNPs.

[0053] The examples contained in the present application include methods for screening and/or diagnosing schizophrenic disorders. In summary, the invention provides that if the base T is found at I7G1584T, the subject may be at higher risk for developing schizophrenia than if G is found at this position. If the base G is found at rs1816071, the subject may be at higher risk for developing schizophrenia than if A is found at this position. If the base C is found at rs1816072 and/ or rs194072 and/ or rs187269, the subject may be at higher risk for developing schizophrenia than if T is found at this position. Likewise, if the base C is found at position rs252944, the subject may be at a higher risk for developing schizophrenia than if G is found at this position.

[0054] Referring to Figure 3, a schematic diagram presenting the distribution of exons in the genome nucleotide sequence of the human *GABRB2* gene is provided. The figure depicts the locations of the SNP sites I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 in *GABRB2*, which can be used in an embodiment of the present invention for determining if an individual has a predisposition for schizophrenia.



1 [0055] The above mentioned polymorphisms can be used in diagnostic tests in order to  
2 identify individuals who have a predisposition for developing schizophrenia. Such diagnostic  
3 tests may utilize a number of technologies known in the art including, use of DNA chip methods  
4 (such as GenFlex<sup>TM</sup> Tag, Affymetrix) and DNA immobilization on beads (such as SIGNET<sup>TM</sup> Y-  
5 SNP Identification System, Marligen Bioscience, Inc.), ABI Prism<sup>®</sup> SNPAPshot<sup>TM</sup> Multiplex  
6 System (Applied Biosystems Inc.) and Pyrosequencing<sup>TM</sup> (Pyrosequencing AB). Diagnostic  
7 tests can also be conducted using allele specific primers, allele specific probes, directing  
8 sequencing of the intron of interest, and denaturing gradient gel electrophoresis. The present  
9 invention therefore provides a method of diagnosing an individual with schizophrenia, or an  
10 individual with a predisposition to schizophrenia, by determining the presence or absence of a  
11 *GABRB2* haplotype. Such haplotype would include the SNP sites I7G1584T, rs1816071,  
12 rs1816072, rs194072, rs252944 and rs187269.

13 [0056] In yet another aspect of the present invention, synthetic or recombinant DNA  
14 molecules are provided for diagnostic purpose. Such molecules comprise a sequence  
15 encompassing one or two or three of the SNPs I7G1584T, rs1816071, rs1816072, rs194072,  
16 rs252944 and rs187269, in one or both their allelic forms, and their flanking regions, and any  
17 haplotype or haplotypes associated with one or more of these six SNPs, as well as any genetic  
18 locus or loci in linkage disequilibrium with the six SNPs, with respect to one or both DNA  
19 strands of the *GABRB2* gene.

20 [0057] In an additional embodiment of the present invention, a method is provided for  
21 screening therapeutic drugs for treatment of schizophrenia, based on the correlation between the  
22 novel SNP I7G1584T, the SNPs rs1816071, rs1816072, rs194072, rs252944, and rs187269, and  
23 the disease. Because these six SNPs are located in the intron regions of *GABRB2*, they may  
24 modulate the synthesis mRNA splicing and the activity of GABA<sub>A</sub> receptors containing the  $\beta_2$   
25 subunit. To detect such modulation, a *GABRB2* gene containing different combinations of the  
26 different allelic forms of these six SNPs may be cloned, and expressed along with other GABA<sub>A</sub>  
27 subunit genes, by means of appropriate expression vectors in cell lines such as P12 (ATCC, The  
28 Global Bioresource Center<sup>TM</sup>), and using commonly used transfection kits such as the CellPfect  
29 Transfection Kit (Amersham Pharmacia Biotech, Inc., USA), and ProFection<sup>®</sup> Mammalian

1 Transfection Systems (Promega Corporation). The cell line may or may not be of neuronal  
2 origin. Transfection efficiency can be enhanced by using a number of reagents known in the art  
3 including, for example, calcium phosphate (Life Technologies Inc.).

4 [0058] In the event that one or more of the SNP's I7G1584T, rs1816071, rs1816072,  
5 rs194072, rs252944 and rs187289, in their schizophrenia-associated allelic form, should alter the  
6 expressed activity of GABA<sub>A</sub> receptor through a modification of the short form/long form ratio,  
7 some structural feature, or the amount of the expressed  $\beta_2$  subunit, the above mentioned cellular  
8 system also may be employed to screen and search for compounds that can restore the normal  
9 level of expressed activity of GABA<sub>A</sub> receptor. Such compounds may be potentially useful  
10 candidate drugs for the treatment of schizophrenia.

11 [0059] The invention further provides kits for detection of SNP sites associated with  
12 schizophrenia.

13 [0060] To detect the SNPs of interest by DNA sequencing or other genotyping methods, a  
14 PCR is performed to amplify the DNA segment of a test subject containing one or more of the  
15 SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, and the PCR  
16 products are subjected to DNA sequencing or other genotyping methods such as single-base  
17 extension or microarray-based sequence-specific oligonucleotide probe hybridization, to  
18 determine the allelic form or forms of the polymorphism(s). The results may be analysed by  
19 computer software packages such as POLYPHRED™ and CONSED™ for DNA sequencing  
20 data, or other suitable software. A kit that may serve such detection may contain, for example,  
21 one or more of the following components including: PCR primers, allele specific probes, DNA  
22 polymerase, PCR reaction buffer, magnesium chloride and four deoxynucleotides for use in a  
23 PCR reaction or extension of probe sequences. In addition, if a sequencing reaction is conducted  
24 a kit may also include, sequencing primers, DNA polymerase, four labelled dideoxynucleotides,  
25 and four un-labelled deoxynucleotides.

26 [0061] In cases where genotyping methods do not require PCR amplification of DNA are  
27 employed, the DNA of a test subject can be directly used in experimental characterization of the  
28 six SNPs, and the haplotype or haplotypes associated with the six SNPs.

1 [0062] The present invention also provides a method of drug screening comprising the steps  
2 of:

3 [0063] (a) Transfecting a vector into a eukaryotic expression system, the vector containing  
4 the DNA sequence corresponding to part or all of the human *GABRB2* gene, by itself or along  
5 with other genes encoding the proteins of the human GABA<sub>A</sub> receptor;

6 [0064] (b) Expressing the vector in the cellular expression system;

7 [0065] (c) Adding a drug to be screened into the cellular system

8 [0066] (d) Analyzing the gene expression of the *GABRB2* gene encoding the GABA<sub>A</sub>  
9 receptor  $\beta_2$  subunit, the *GABRB2* gene being in all possible allelic combinations relating to the  
10 SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, and also analysing  
11 the expression and resultant activity of the GABA<sub>A</sub> receptor in the cellular system.

12 [0067] (e) Determining the effect of chemical compounds or preparations on the cellular  
13 system with respect to the expression of GABA<sub>A</sub>  $\beta_2$  subunit and the GABA<sub>A</sub> receptor, and the  
14 activity of the expressed GABA<sub>A</sub> receptor in the cellular system.

15 [0068] The chemical compounds or preparations so identified are potentially useful  
16 candidate drugs for the treatment of schizophrenia.

17 [0069] While the novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072,  
18 rs252944 and rs187269 may be used singly or jointly for diagnostic or drug development  
19 purposes, one or more genetic markers belonging to the same haplotype as, or in linkage  
20 disequilibrium with, one or more of these six SNPs, may also be employed for these purposes.

21 [0070] Furthermore, while the PCR and DNA sequencing methods described below in  
22 Example 1, and similar methods such as DNA sequencing by means of pyrosequencing  
23 (Pyrosequencing AB), can reveal the genotype of a test DNA sample in terms of the SNPs  
24 I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, alternative methods can  
25 also be employed for this purpose. Such alternate methods include the use of DNA chip methods  
26 (such as GenFlex<sup>TM</sup> Tag, Affymetrix) and DNA immobilization on beads (such as SIGNET<sup>TM</sup> Y-  
27 SNP Identification System, Marligen Bioscience, Inc.), instead of DNA sequencing, and methods  
28 using single DNA molecule sequencing instead of PCR (such as ABI Prism<sup>®</sup> SNPshot<sup>TM</sup>  
29 Multiplex System, Applied Biosystems Inc.).

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[0071] The examples presented below are provided to illustrate the present invention and are not meant to limit the scope of the invention as will be apparent to persons skilled in the art.

[0072] **EXAMPLE 1: Identification of the Novel SNP site of the Invention:**

[0073] The novel SNP I7G1584T of the invention was identified in the method as described below.

[0074] For discovery of SNPs within the region of interest, specific primers were designed in an adjacent-primer-overlapping-200-base-pair manner by using the online primer designation program, Primer3™ ([http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi)). The specificity of each suggested primer to the human genome was checked through BLAST™ (National Center for Biotechnology Information - <http://www.ncbi.nlm.nih.gov/BLAST>). Only both forward and reverse primers with less than 5 hits to human genome were considered as specific primers and employed.

[0075] PCR was performed in a final volume of 20  $\mu$ l containing: 10 ng sample DNA from either controls or patients; 0.075  $\mu$ mol/L of each primer; 500  $\mu$ mol/L of each dNTP; 2.5 mmol/L  $MgCl_2$ ; and 1U *Taq*™ polymerase (Amersham Pharmacia Biotech, Inc, USA). Gradient PCR was engaged initially to determine the optimum annealing temperature for each pair of primers. Examples of primer sequences and their optimum annealing temperatures are listed in Table 3. PCR amplification consisted of denaturation at 94°C for 5 min followed by 40 cycles of 1 min at 94°C, 1 min at optimum annealing temperature for each pair of primers to DNA template, 1.5 min at 72°C and a final extension step at 72°C for 5 min. PCR products were then resolved using 1.5% agarose gel electrophoresis and staining with ethidium bromide to confirm desired and specific products generated. PCR products were purified by MultiScreen™ PCR<sub>96</sub> Purification kit (Millipore Corporation, Bedford, USA).

[0076] Discovery of SNPs within the region of interest were performed by auto-sequencing of PCR fragments on both strands. The sequencing reactions contained: 2  $\mu$ l BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems); 5  $\mu$ l purified PCR products; and 1 mmol/L of primer. Sequencing cycling conditions consisted of 1 min denaturation at 96°C followed by 34 cycles of 96°C for 30sec, 50°C for 30sec and 60°C for

3min. Sequencing products were purified by AutoSeq96™ Plates containing DNA Grade Sephadex™ G-50 (Amersham Pharmacia Biotech, Inc, USA). Purified sequencing products were then denatured at 95°C for 5 min with addition of 5µl Hi-Deionized Formamide. Denatured sequencing products were run on the ABI Prism model 3100 DNA sequencer according to ABI protocols.

[0077] Sequence data were aligned and analyzed for SNPs by POLYPHRED™ software (Nickerson DA, Tobe VO & Talyor SL, 1997 Nucleic Acids Res. 15; 25: 2745-51) and CONSED™ software (Gordon D, Abajian C and Green P, 1998 Genome Res. 8: 195-202).

#### **EXAMPLE 2: Detection of Nucleotide Sequences Associated with Schizophrenia**

[0078] This example describes the protocol utilized to find and test for the presence of one or more the above-mentioned SNPs. In this example, PCR-amplified products are synthesized to encompass the target regions of human GABA<sub>A</sub> receptor  $\beta_2$  subunit of the *GABRB2* gene. A pair of primers, specific to each of these regions, and which can be used for amplification, are shown in Table 3.

**Table 3 The Primers of  $\beta_2$  Subunit Genes for Both PCR and DNA Sequencing**

Cluster ID of dbSNP	Forward primer	Reverse primer	Optimum annealing temperature for PCR
I7G1584T	atggaggaaaggtccatatctagt (SEQ ID NO:12)	cctctaagctgtaatcggaaggta (SEQ ID NO:13)	62°C
rs1816071	atggaggaaaggtccatatctagt (SEQ ID NO:10)	cctctaagctgtaatcggaaggta (SEQ ID NO:11)	62°C
rs1816072	atggaggaaaggtccatatctagt (SEQ ID NO:8)	cctctaagctgtaatcggaaggta (SEQ ID NO:9)	62°C
rs194072	taccttccgattacagcttagagg (SEQ ID NO:2)	tggagaggactctaggtcaacttt (SEQ ID NO:3)	64°C
rs252944	cacaatgattttccgagaccat (SEQ ID NO:4)	ttgtaaagctattgtccagcaagt (SEQ ID NO:5)	62°C
rs187269	agcacttgctgcactaacagaata (SEQ ID NO:6)	agacaatgcctaattgtcctctgg (SEQ ID NO:7)	62°C

**[0079] PCR Amplification of SNP Containing Regions and Sequencing of Amplified Regions**

**[0080]** The following reagents were used for amplification reactions for amplifying the regions of interest: 2  $\mu$ l of PCR buffer (Amersham Pharmacia Biotech, Inc, USA); 2.5 mM  $MgCl_2$ ; 500  $\mu$ M dNTPs; 0.075  $\mu$ M of each primer; 10 ng DNA; and 1 U Taq™ DNA polymerase (Amersham Pharmacia Biotech, Inc., USA); in a total volume of 20  $\mu$ l.

**[0081]** PCR amplification was performed in the PTC-200™ Peltier thermal-cycler (MJ Research, Inc.) and RoboCycler™ (Stratagene, USA) using 96-well microplates under the following thermal cycle conditions: 5 minutes at 94 °C for denaturation; and 40 cycles for optimal DNA amplification that entailed denaturation at 94 °C for 30 seconds, annealing at 60 °C – 62 °C for 30 seconds, and polymerization at 72 °C for 1.5 minutes.

**[0082]** The PCR products were purified using a MultiScreen®-PCR kit, (Millipore Corporation, Bedford, USA), and were directly sequenced using a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) under the following conditions: 96 °C for 1 minute followed by 34 cycles of 96 °C for 30 seconds, 50 °C for 15 seconds, and 60 °C for 3 minutes.

**[0083]** Sequencing products were purified by AutoSeq96™ Plates containing DNA Grade Sephadex™ G-50 (Amersham Pharmacia Biotech, Inc, USA). Purified sequencing products were then denatured at 95°C for 5 min with addition of 5 $\mu$ l Hi-Deionized Formamide. Denatured sequencing products were run on the ABI Prism model 3100 DNA sequencer according to ABI protocols. Sequence data were aligned and analyzed for SNPs by POLYPHRED™ software (Nickerson DA, Tobe VO & Talyor SL, 1997 Nucleic Acids Res. 15; 25: 2745-51) and CONSED™ software (Gordon D, Abajian C and Green P, 1998 Genome Res. 8: 195-202). Each PCR product was sequenced from both ends using the same primers as those utilized for the DNA amplification described above.

**[0084]** The DNA sequencing results using this procedure are shown in Table 1. This table shows the nature of the SNP bases in samples from control (CON) and schizophrenic (SCH) subjects in term of the allelic form of the six SNPs examined.

[0085] Although one method of detecting the six polymorphic sites associated with schizophrenia is provided above, it will be understood by persons skilled in the art that other detection methods may also be utilized. For example, detection can also be accomplished, inter alia, through allele specific probes designed to identify the presence or absence of an individual SNP site, allele specific primer extension reactions, and single base primer extension reactions.

[0086] **EXAMPLE 3: Genotyping of I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269 polymorphisms**

[0087] For the novel SNP I7G1584T, and the five SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269, the DNA sequencing results in Table 1 from healthy Controls, and Schizophrenics satisfying DSM IV criteria (American Psychiatric Association, 1994) were analysed by genotyping in order to determine whether the aforementioned SNPs were associated with an increased risk of schizophrenia. Identification of SNPs was conducted using the primers and sequencing method as described in Example 2.

[0088] **Table 1: Forms of SNPs in the GABRB2 Gene**

Cluster ID of dbSNP	Allele			Genotype		
		CON	SCH		CON	SCH
I7G1584T	G	163	185	G/G	72	72
	T	21	55	G/T	19	41
				T/T	1	7
	Total	184	240	Total	92	120
rs1816071	A	137	142	A/A	56	51
	G	37	82	A/G	25	40
				G/G	6	21
	Total	174	224	Total	87	112

rs1816072	T	131	125	T/T	44	37
	C	71	105	T/C	43	51
				C/C	14	27
	Total	202	230	Total	101	115
rs194072	T	173	184	T/T	77	73
	C	23	56	T/C	19	38
				C/C	2	9
	Total	196	240	Total	98	120
rs252944	G	240	148	G/G	104	55
	C	36	52	G/C	32	38
				C/C	2	7
	Total	276	200	Total	138	100
rs187269	T	284	188	T/T	117	70
	C	58	74	T/C	50	48
				C/C	4	13
	Total	342	262	Total	171	131

[0089] As shown in Table 2, the Chi-square test for comparing the genotypes of Controls (CON) and Schizophrenics (SCH) shows a very significant increase of homozygote T/T carriers of novel SNP I7G1584T, homozygote G/G carriers of rs1816071 and homozygote C/C carriers of rs1816072, rs194072, rs252944 and rs187269 among schizophrenics. The comparison of allele frequencies also indicates a highly significant increase in T of novel SNP I7G1584T, G of rs1816071 and C of rs1816072, rs194072, rs252994 and rs187269 in Schizophrenics in comparison to Controls.

[0090] Table 2 shows the frequency distribution of genotypes and alleles for the novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269 polymorphisms. Table 2 shows the *p* values from the Chi-square test, odds ratios (OR) and 95%



confidence interval (95% CI) in comparing the following samples: a) 120 Schizophrenics (SCH) and 92 Controls (CON) for novel SNP I7G1584T; b) 112 Schizophrenics and 87 Controls for rs1816072; c) 115 Schizophrenics and 101 Controls for rs1816072; d) 120 Schizophrenics and 98 Controls for rs194072; e) 100 Schizophrenics and 138 Controls for rs252944; and f) 131 Schizophrenics and 171 Controls for rs187269. All the Controls and Schizophrenics were *Han* Chinese. The statistic software Prism3™ version 3.02 was used for allele and genotype significance testing.

[0091] **Table 2: Association of SNPs in the *GABRB2* Gene with Schizophrenia.**

	Allele					
Cluster ID of dbSNP	I7G1584T	rs1816071	rs1816072	rs194072	rs252944	rs187269
<i>p</i> Value	0.0022	0.0009	0.0266	0.0018	0.0003	0.0009
OR	2.308	2.138	1.55	2.289	2.342	1.927
95% CI	1.338-3.981	1.358-3.366	1.051-2.285	1.350 - 3.882	1.460-3.755	1.305-2.847
	Genotype					
Cluster ID of dbSNP	I7G1584T	rs1816071	rs1816072	rs194072	rs252944	rs187269
<i>p</i> Value	0.011	0.0109	0.1044	0.0125	0.0018	0.0031
OR	--	--	--	--	--	--
95% CI	--	--	--	--	--	--

[0092] Table 4 below shows the estimated frequencies for certain haplotypes of the six SNPs and the significance of their association with schizophrenia (SCH) in comparison to unaffected controls (CON). Maximum likelihood haplotype frequencies between possible SNP haplotypes

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1 were estimated by the Expectation-Maximization algorithm (Slatkin M, Excoffier L, 1996).  
 2 Testing for linkage disequilibrium in genotypic data using the expectation-maximization  
 3 algorithm. *Heredity* 76:377-383) using the program SNPHAP™  
 4 (<http://www.hgmp.mrc.ac.uk/Menu/Help/snphap>) under Hardy-Weinberg assumption of gamete  
 5 independence. Association analyses were performed by comparing the haplotype frequencies of  
 6 Controls and Schizophrenics through the Chi-square test. Association of schizophrenia and  
 7 estimated two-loci and six-loci haplotype were accessed by CLUMPT™  
 8 (<http://www.hgmp.mrc.ac.uk/Registered/Help/clump/>) (Sham PC and Curtis D, 1995. Monte  
 9 Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum*  
 0 *Genet* 59: 97-105) to yield the Chi-square value and its corresponding *p*-value.  
 1 [0093] The novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072 and  
 2 rs252944 SNP are located between Exons 7 and 8, and the rs187269 SNP is located between  
 3 Exon 8 and 9. Exon 9 is present in the long form variant of the  $\beta_2$  subunit of GABA<sub>A</sub> receptor  
 4 mRNA but absent from the short form variant. These six SNPs are thus located close to a region  
 5 that may play a role in modulating the properties and/or quantity of  $\beta_2$  incorporated into GABA<sub>A</sub>  
 6 receptors, including but not limited to the alternative splicing of the *GABRB2* mRNA to yield the  
 7 short form versus the long form of  $\beta_2$ .

8  
 9 **Table 4: Haplotype Frequencies and Significance of Association with Schizophrenia**  
 10

Haplotype	Frequency %		$\chi^2, df^*$	<i>P</i> (OR; 95%CI)
	CON	SCH		
I7G1584T-rs1816071			16.95, 3	0.00074
G-A	76.81	59.85		
G-G	11.78	18.02		
T-A	0.00	3.75		
T-G	11.41	18.38		
T-G vs. G-A + G-G + T-A			3.70, 1	0.054 (1.725; 0.9855-3.019)
I7G1584T-rs187269			12.89, 3	0.0049
G-T	81.70	67.38		
G-C	6.9	11.36		
C-T	1.45	3.10		
C-C	9.95	18.16		
C-C vs. GT + G-C + C-T			6.738, 1	0.0094 (2.04; 1.182-3.523)
rs1816071-rs1816072			17.44, 3	0.000583

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A-T	66.71	48.02		
A-C	12.36	15.76		
G-T	1.31	6.16		
G-C	19.62	30.07		
G-C vs. A-T + A-C + G-T			5.44, 1	0.0197 (1.733; 1.089-2.758)
rs1816071-rs187269			22.90	0.000043
A-T	75.0	55.13		
A-C	2.51	9.55		
G-T	8.98	13.73		
G-C	13.51	21.59		
G-C vs. A-T + A-C + G-T			5.45	0.0197 (1.774, 1.092-2.883)
rs1816072-rs194072			12.73, 3	0.0053
T-T	63.55	52.89		
T-C	0.78	1.72		
C-T	23.95	21.97		
C-C	11.71	23.42		
C-C vs. T-T + T-C + C-T			11.45, 1	0.00072 (2.284; 1.404-3.716)
rs194072-rs252944			8.39, 3	0.03868
T-G	85.23	73.85		
T-C	0.38	1.24		
C-G	0.46	1.03		
C-C	13.92	23.89		
C-C vs. T-G + T-C + C-G			6.77, 1	0.009281 (1.906; 1.167-3.112)
rs252944-rs187269			26.24, 3	0.000009
G-T	80.10	69.45		
G-C	6.79	7.76		
C-T	4.18	0.72		
C-C	8.93	22.06		
C-C vs. G-T + G-C + C-T			20.87, 1	0.000005 (2.960; 1.829-4.808)
rs194072-rs187269			26.83, 3	0.000007
T-T	78.62	68.01		
T-C	5.96	8.36		
C-T	5.30	0.64		
C-C	10.12	22.98		
C-C vs. T-T + T-C + C-T			17.89, 1	0.000024 (2.624; 1.660-4.148)
rs194072-rs252944-rs187269			30.72, 7	0.000072
T-T-T	80.39	65.17		
T-C-C	5.54	7.55		
T-C-T	0.00	0.00		
T-C-C	0.00	1.09		
C-T-T	0.00	0.00		
C-T-C	0.45	1.08		
C-C-T	4.89	1.19		
C-C-C	8.36	23.89		
C-C-C vs others			17.21, 1	0.000034 (2.857; 1.713-4.766)
I7G1584T-rs194072-rs252944-rs187269			29.29, 15	0.015
G-T-T-T	81.97	65.69		
G-T-T-C	09.13	7.09		
G-T-C-T	0.00	0.00		
G-T-C-C	0.00	1.23		

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G-C-T-T	0.00	0.81		
G-C-T-C	0.00	1.50		
G-C-C-T	1.85	0.02		
G-C-C-C	0.59	1.25		
T-T-T-T	0.00	1.33		
T-T-T-C	0.00	0.00		
T-T-C-T	0.00	0.00		
T-T-C-C	0.00	0.00		
T-C-T-T	0.00	0.00		
T-C-T-C	0.00	0.00		
T-C-C-T	2.18	1.40		
T-C-C-C	4.29	19.68		
T-C-C-C vs others			12.79, 1	0.0003 (4.860; 1.888-12.51)

Note: df= degree of freedom

**[0094] EXAMPLE 4: Method for Screening Enhanced Susceptibility to Schizophrenia and for Genotyping Schizophrenics**

**[0095]** Example 2 describes a method for determining the nature of the novel SNP I7G1584T, and the SNPs rs1816071, rs1816072, rs194072, rs252944 and rs187269 in Control subjects and Schizophrenic patients. Example 3 shows that a base T in SNP I7G1584T, a base G in rs1816071 and a base C in rs1816072, rs194072, rs252944 and rs187269 signifies an increased risk of schizophrenia. Furthermore, these six SNPs provide a basis for screening for enhanced susceptibility for schizophrenia, and genotyping schizophrenia patients with the aim of applying pharmacogenetics to determine the optimal therapeutic to be employed for their treatment.

**[0096]** The same procedures employed in Example 2 also can be performed on test subjects with unknown susceptibility to schizophrenia or schizophrenic patients with unknown genotype. In either case, the procedures employed will reveal the genotype of the test DNA sample with respect to the bases at the SNP positions the novel SNP I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269. The resultant genotype in turn will indicate whether the test subject carries enhanced genetic susceptibility to schizophrenia. In the case of a patient known to be a schizophrenic, the genotype will provide a basis for diagnostic classification and for pharmacogenetic profiling towards achieving optimizing therapy.

**[0097]** To perform genotyping using the PCR and sequencing procedures as described in Example 2, the following sequences, or sequences similar to them, will be required.

**[0098]     Description of Sequence Listings**

**[0099]     SEQ ID NO:1:** The native DNA sequence of Exon 7, Intron 7, Exon 8, Intron 8 and Exon 9 of the human *GABRB2* gene, with variation noted at positions 1584, 1803, 2103, 3106 and 3424 of intron 7, and position 1265 of Intron 8

**[00100]    SEQ ID NO:2** is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 3106 of Intron 7, corresponding to rs194072, of the human *GABRB2* gene.

**[00101]    SEQ ID NO:3** is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 3106 of Intron 7, corresponding to rs194072, of the human *GABRB2* gene.

**[00102]    SEQ ID NO:4** is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 3424 of Intron 7, corresponding to rs252944, of the human *GABRB2* gene.

**[00103]    SEQ ID NO:5** is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 3424 of Intron 7, corresponding to rs252944, of the human *GABRB2* gene.

**[00104]    SEQ ID NO:6** is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 1265 of Intron 8, corresponding to rs187269, of the human *GABRB2* gene.

**[00105]    SEQ ID NO:7** is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 1265 of Intron 8, corresponding to rs187269, of the human *GABRB2* gene.

**[00106]    SEQ ID NO:8** is the nucleotide sequence for the forward primer used for the amplification of the SNP at position 2103 of Intron 7, corresponding to rs1816072, of the human *GABRB2* gene.

**[00107]    SEQ ID NO:9** is the nucleotide sequence for the reverse primer used for the amplification of the SNP at position 2103 of Intron 7, corresponding to rs1816072, of the human *GABRB2* gene.

1 [00108] SEQ ID NO:10 is the nucleotide sequence for the forward primer used for the  
2 amplification of the SNP at position 1803 of Intron 7, corresponding to rs1816071, of the human  
3 *GABRB2* gene.

4 [00109] SEQ ID NO:11 is the nucleotide sequence for the reverse primer used for the  
5 amplification of the SNP at position 1803 of Intron 7, corresponding to rs1816071, of the human  
6 *GABRB2* gene.

7 [00110] SEQ ID NO:12 is the nucleotide sequence for the forward primer used for the  
8 amplification of the SNP at position 1584 of Intron 7, corresponding to I7G1584T, of the human  
9 *GABRB2* gene.

10 [00111] SEQ ID NO:13 is the nucleotide sequence for the reverse primer used for the  
11 amplification of the SNP at position 1584 of Intron 7, corresponding to I7G1584T, of the human  
12 *GABRB2* gene.

13 [00112] The various publications and published methods mentioned above are incorporated  
14 herein by reference.

15 [00113] Although the invention has been described with reference to certain specific  
16 embodiments, various modifications thereof will be apparent to those skilled in the art without  
17 departing from the spirit and scope of the invention as outlined in the claims appended hereto.

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**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. An isolated polynucleotide having a nucleotide sequence comprising corresponding to SEQ ID NO: 1, said polynucleotide comprising a portion of the human *GABRB2* gene including a polymorphic site at position 1584 of intron 7.
2. The isolated polynucleotide of claim 1 wherein the nucleotide at position 1584 of Intron 7 is selected from G or T.
3. An isolated polynucleotide having a nucleotide sequence which is complementary to the polynucleotide of claim 2.
4. A PCR primer set for amplifying regions of a polynucleotide corresponding to SEQ ID NO: 1, said primer set comprising a first primer having a sequence corresponding or complementary to a sequence corresponding to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 or SEQ ID NO: 12, and a second primer having a sequence corresponding or complementary to a sequence corresponding to SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11 or SEQ ID NO: 13.
5. A method of predicting whether an individual is more likely to suffer from schizophrenia comprising the identification of DNA sequences corresponding to one or more SNPs selected from the group consisting of: SNPs at positions 1584, 1803, 2103, 3106, and 3434 of intron 7, and position 1265 of intron 8 of the human *GABRB2* gene on one or two of the individual's chromosomes, or other genetic markers in the same haplotype as, or in linkage disequilibrium with, one or more of said SNPs.
6. The method of claim 5 wherein the presence of a C at position 3106 of Intron 7 indicates that the individual is more likely to suffer from schizophrenia.



7. The method of claim 5 wherein the presence of a C at position 3424 of Intron 7 indicates that the individual is more likely to suffer from schizophrenia.
8. The method of claim 5 wherein the presence of a C at position 1265 of Intron 8 indicates that the individual is more likely to suffer from schizophrenia.
9. The method of claim 5 wherein the presence of a C at position 2103 of Intron 7 indicates that the individual is more likely to suffer from schizophrenia.
10. The method of claim 5 wherein the presence of a G at position 1803 of Intron 7 indicates that the individual is more likely to suffer from schizophrenia.
11. The method of claim 5 wherein the presence of a T at position 1584 of Intron 7 indicates that the individual is more likely to suffer from schizophrenia.
12. A method of genotyping an individual in order to determine a clinical and pharmacogenetic classification of a schizophrenia-spectrum disorder of the individual suffering from such disorder, said method comprising the identification of DNA sequences corresponding to one or more SNPs selected from the group consisting of: SNPs at positions 1584, 1803, 2103, 3106, and 3434 of intron 7, and position 1265 of intron 8 of the human *GABRB2* gene on one or two of the individual's chromosomes, or other genetic markers in the same haplotype as, or in linkage disequilibrium with, one or more of said SNPs.
13. The method of claim 12 wherein the presence of a C at position 3106 of Intron 7 indicates that the schizophrenia-spectrum disorder of the individual has a genotype that includes the presence of a C at position 3106 of Intron 7 of *GABRB2*

14. The method of claim 12 wherein the presence of a C at position 3424 of Intron 7 in either one or two chromosome indicates that the schizophrenia-spectrum disorder of the patient has a genotype that includes the presence of a C at position 3424 of Intron 7 of *GABRB2* in either one or two chromosomes.
15. The method of claim 12 wherein the presence of a C at position 1265 of Intron 8 in either one or two chromosome indicates that the schizophrenia-spectrum disorder of the patient has a genotype that includes the presence of a C at position 1265 of Intron 8 of *GABRB2* in either one or two chromosomes.
16. The method of claim 12 wherein the presence of a C at position 2103 of Intron 7 in either one or two chromosome indicates that the schizophrenia-spectrum disorder of the patient has a genotype that includes the presence of a C at position 2103 of Intron 7 of *GABRB2* in either one or two chromosomes.
17. The method of claim 12 wherein the presence of a G at position 1803 of Intron 7 in either one or two chromosome indicates that the schizophrenia-spectrum disorder of the patient has a genotype that includes the presence of a G at position 1803 of Intron 7 of *GABRB2* in either one or two chromosomes.
18. The method of claim 12 wherein the presence of a T at position 1584 of Intron 7 in either one or two chromosome indicates that the schizophrenia-spectrum disorder of the patient has a genotype that includes the presence of a T at position 1584 of Intron 7 of *GABRB2* in either one or two chromosomes.
19. A method of drug screening for identifying drugs for treating schizophrenic disorders, the method comprising:
  - a) transfecting a vector into a eukaryotic expression system, said vector containing a DNA sequence corresponding to at least a portion of the human *GABRB2* gene, said portion of

the *GABRB2* gene including one or more SNPs chosen from the group consisting of: I7G1584T, rs1816071, rs1816072, rs194072, rs252944, and rs187269;

- b) expressing said vector in a cellular expression system;
- c) adding a drug to be screened into said cellular system;
- d) analyzing the expression of the *GABRB2* gene encoding GABA<sub>A</sub> receptor  $\beta_2$  subunit and also analysing the expression and resultant activity of the GABA<sub>A</sub> receptor in the cellular system;
- e) determining the effect of said drugs on the expression of the GABA<sub>A</sub>  $\beta_2$  subunit and the GABA<sub>A</sub> receptor, and the activity of the expressed GABA<sub>A</sub> receptor in the cellular system; and,
- f) identifying drugs capable of altering the levels of expression of different forms of *GABRB2* gene containing different alleles of one or more of the six SNPs I7G1584T, rs1816071, rs1816072, rs194072, rs252944 and rs187269, and genetic markers belonging to the same haplotype or haplotypes of , or in linkage disequilibrium with, one or more of these six SNPs.

20. A diagnostic kit for detecting the identity of SNPs at positions 1584, 1803, 2103, 3106, and 3434 of intron 7, and position 1265 of intron 8 of the human *GABRB2* gene, said kit comprising components for the determination of said SNPs.

Intron 7 Base No.

53. TTGATAATGATGATGTTCTAAAAATGCTTCTAGGTTCTATCCAGGTTATCCCTCAG  
 113. CTTTAGCTTAAAGCAAAATGGCTACTTTATCCCTGCAAAATACATGCTTCCATCTT  
 173. GATTACCATCTCTCCCTGGGTCTCTCTCTGGGATTAAATACGATGCTCAGCTGGCAAGGT  
 233. GGCATTAGGTAGGCCATTTTATTACTGCTGTGTGGATTGTGACTCTGTGTGTACATTTCTG  
 293. TGTTCGCATGCACGCTTTTCCCTATCCGAAATTTGTGGTGATGACGCAACTGAACCT  
 353. CTCACACTGTAAGAGTTTCGCTATGAGACTTTGGGTGATCTGATTCAGCATTAATCAAT  
 413. TAAACACGAAGTAAAGCTCTTTCATGTCACTATTAGTTAAACGATATCTGTGTGATAATG  
 473. AGATTAGTCCCATCAGTTTGATATTTTATTTGAAACATATTTCATATAGTATTGCTTGCA  
 533. TAATTGTTCAATTTGTATCTGTGCTTCTTACCTAAGAAGACCTCCCTTCACTGTTTTT  
 593. TCTCCATGAAAGACAAGAAGATGTTGTGTGTAGCAATGGATAAGCAGAAAGAAATCTAA  
 653. AGTCTGTGTTAAACATTCAGAGTAGCATGACTAAGGCAGGAGACAACAAAGTAAAGATATT  
 713. GAGAAGTAATCTGTGGCATCTCTCATCTTATGCTGTTTTTTTCTAACAAACACAAAGGA  
 773. GAATAAATTACATTTTATGAACATTTTGTATTAACTTCAGATAAAGTAAATTTATGGC  
 833. CTTTACACGATCTTGATTATATGGAATCTGACTATTCCCTTTAAATGTTGAATGTT  
 893. CACTTTTACCATTAGATCTCAATTCATCATTTCTTTCTAGAAGAGCCACTCATTTATT  
 953. ATTCAACAGTAATTTGTTAAATACCTAATATATGCTAATTTGTAAGCACTGGAGATAG  
 1013. AGCAGTCTTCTGAAAGTTATATCTAGCAGTAAAGATATACAATAAATTTAATAACCACA  
 1073. TAAATTATACATGTGGTAAATCTATAGAGGGATATAAAGCAAGGGGATGGGTTTGGCT  
 1133. AGAAGTTCAGTTTAGAATAATTAGGGCCGACTTCCTCAGAAGTTGTCTTGGAACTTAAG  
 1193. AAGAGGATTGAGAGCAATGTTGGATACCTGGGTGATAATATTCAGATGACTGCAAGG  
 1253. CCGTGGGTAGGAACAAACCAACAGTCTCAGCAGGAGGCTAATGAGGCCAAGTGAGTTTCA  
 1313. GGGAGTAGCAAGATAATGTTAGGGAAAGAGGCCCTAAAGGATCACAAGGGGCTATTAC  
 1373. AAAGGGATGGTAGGCCACGACAAAGGCTTTGCTTTTATTGTAATGAATAGCCATTGAC  
 1433. TGAGCATAAAGTGGCATGATTGATTCTGTTTTAAAGGGATCACTTCGGCTACTGGGT  
 1493. TAAATTAAATATGTGGCTCAAGGATGGGAGGAAGAGAGAATGACCTCTCAGGCTG  
 1553. AGTCAAGCACCTGTGATATATACACTTGCAGTACCATAGACCTCCCTGTGTGTAGACTA  
 1613. ACCACAGTTATAACTTTTGTCTTCTAGTGGTGTGATGCTCTTTCCCTTCAGCAATGA  
 1673. AAGATCCATGGAGGAAGGTCATATCTAGTTTATATCTGCAGTGTCTTGCATGGGAA  
 1733. TGGTGTCTCAGTAAACATTTGCTGAATGAAGAAGGAATAAAATTTGCAATATCCCATTT  
 1793. TTCCAAAGTTGAACATGTCTTTTGTATCTGATTACAGGAACATAAATAATTCATTAA  
 1853. CTTTTAGAAGACATTTTAAATGTCTTTATGTGAGCAATTTATTACTAGTTTCAGAAA  
 1913. TACTTTAAATGAGATTTAGATAAGTAGTAAATCAGCAATGTGAGTTTATTATATACG  
 1973. TATGATACAGGATCATCCGTAGTTGAATGATGAAGAGAAAATGAAACTGGAAAATGTC  
 2033. TCAAAATGACAGCTATAAAGAGGACACGAGAAAATGTAAGATACCTGTATCTAAAGCAAGA  
 2093. CCAAGGTTTATGGGCGTGTCTCAGAAATCAATGCTCAGATTTATTTTTTATCTGTAGC  
 2153. TGTCTCTGGTGGGATATAAAACAAATAGAACTATGACATTTATTACTACTGAGATAA  
 2213. AATTAAATCAGAAAACGTGTCTCCCTCCAAATCATAGGAATGAAATTTAACTTCAGGAT  
 2273. GCGTTATTGGATAGTGTTTAAGGGCTGGATTCTCTGAGTCAGAAAGATTTCTATTCCAAT  
 2333. GGCAACTCTTAACCTTTCCATCTGTGTGATCTTGACCTCTGAGCTTTGTCTATTTTCA  
 2393. TCACATTTTGTCTACAAAGCTGGACTTTATCATATTTCAGGACCTTGGACTTTTCAGTGCAT  
 2453. TTACCTTCCGATTACAGCTTAGAGGGAACCTACCTCCCTCTGCAAGAGATACCAACTCA  
 2513. ACAGGACAAACAAATACAGCTGACCCAGTGTCACTTTTCCATAAGCAGTCTAGAAATG  
 2573. CATCTGTGTTATTTATCTAGGCATTATATGCCTAATTTGCTCGATCCATATACATGAGGGC  
 2633. TATGATTCTCTGCTTCTCTGTACCTCCACTCCAAATTTCCAGATGTTTATAATCATTTA  
 2693. TTGATATTGCTGTATAATGTATTCTTTAATTTTCAGTACCTTGTCTGTTATTTAATGA  
 2753. CTTCACTTCCATCATTGAGTTGATTACTCTGAAAACCTCTTAACCTGGCATCTCTTTT  
 2813. TTTTCTTCTTCTTCTGAGACGAGTCTGTGCTGTGGCCAGGCGGAGTGCAGTGGCG  
 2873. GGATCTCGGCTCACTGCAAGCTCCGCTCCCGGTTTCAGCCATTCTCTGCTCAGCCT  
 2933. CCCGAGTAGCTGGGACTACAGGCGCCGCCATCAAGCCCGCTAATTTTGTGATTTT  
 2993. AGTAGAGACAGGTTTACCGTGTAGCCAGGATGCTCTGATCTCTGACCTCTGTGATC  
 3053. CACCCGCTCTCGGCTCCCAAGTGTCTGGGATTACAGCGTGAGCCACCGCGCCCGCCAA  
 3113. CTGGCATTCTTACTCTGCTGCTGCTCTCTCCCATCATCCACACTGTCAACACAGT  
 3173. GATGTTCTAAATGCAAAATGCCCCCTTGTCTGAAGCTGGCCCTTACCTCCAGTCTTC  
 3233. TAATTTTAAGTTCAAACTCCCCATTAGGCTCTCTGATGGCCCTTAACTCCAGTCTTC  
 3293. TCAGGATCTTCCCTCTCTGCTTGTCAATGTACCCACAATGATTTTCCGAGACCATGGT  
 3353. AATGGACTGTGACTCTCCCCAAATCCAACTTGATATAAAGCTCTCTCCACAGCAG  
 3413. TCCCATTAAGTCAAAATGACTTTGACCCCAAGTTCTGGGTTGCCCTGGGTGACCTTCAC  
 3473. TCATTTTTCAGAAATCAGCTCAAAAGTCTCTATTTCTTAACCTCATCCCTGGCACATCAG  
 3533. AAAGTTGACCTAGAGTCTCTCCATATTCTGAACCTTACCTTACACATGTGCCCATCAT  
 3593. AGCATTTAATTCAGTTTAAACCCGCACTAGATATATACTATAAATACAAAATTTAA  
 TTAAGAAGGACATCAACGTTCACTGATCACTGTAAGCTAATTTATACCTATATCCATT  
 CCTATCTAGGCTGGCATGCAATAGTAAAGGCCAATAAATAGATTGTGGCATATATG  
 AATGAGAAAATGAATATAGATCACCTTTCCAGCTATTAGAAGAGAGAAAAGAGATAGGA  
 ACATTATTACTTAACTCTCCCTTTTCTCAGGAATCACTAGTCTCTCAGATGAGC  
 ACAATCAACACCCACTCCGGGAACTCTCCCTAAATCCCTATGTGAAGGCCATTGAC  
 ATGTACCTGATGGGCTCTTGTCTTGTCTTTCATGGCCCTCTGGAATATGCCCTAGTC  
 AACTACTCTCTTGGGAGGGGCCCAACGCAAGAAAGAGCAGCTGAGAGGCTGCC  
 AETGCCAACAATGAGAAGTGGCCTGGATGTCAACAAGGTAATTCAGAGGGCAAGCCC  
 TCCTTGCTCTTAACTCATGGAAGATGTAGCAATACAGGTTGATGACCTTGTGAGC  
 AAATGAGGAACCAAGCCAGGGAAGAGAAATGGGTAATTTCCCAAGGCACACAAACAA  
 TTAATGATGGAAGCACAATTAGGGAACCAACTCATGACACAAGATTGGATGCCCTTTC  
 ATTAACAAGAGGAGTGACCAACATGAGAAGACCTTTATGATGCTTCTCATATTGAG  
 TATAGTACCTATAATTTATGCACTCTTAAATTTATCTCACTAATTTATTTAAAAAG  
 GAAATTTAATGTTGCTTCACTCAGTATTACTTGTGACAAATAGCTTTTACAA

Fig 1a

TTGATAATGATGATGTTCTAAATTCGCTTTCTAGGTTCCATCCAGGTTATCCCTCAG  
 CTTTAAAGCTTAAAGAGAACATTTGGCTACTTTATCCGCAACATACATGCTTCCATCCCT  
 GATTACCATCTCTCTGCTGCTCTCTCTCTGATTAAATACGATGCTTCAGCTGCAAGGGT  
 GGCATTTGGTAGGCTATTTATTTACTGCTTGTGGATTTTGACTCTGTGTGTACATTTCTG  
 TGTTCGCATGCACGCTTTCTCTTATCTCTGAAATTTGTGGTGCATGACGCAACTGAACCT  
 CTCACACTGTAAAGAGTTTCGCTATGAGACTTTGGGTGATCTGATTTCAGCATTAATCAAT  
 TAAACACGAAGTAAAGCTCTTTTCATGTCAGTATTAGTTAACCGTATATCTGTTGATAATG  
 AGATTAGTCCCATCAGTTTGATATTTTATTTGAAACATATTTCATATAGTATTGCTTGCA  
 TAATTGTTCAATTTGTATCTGTGCTCTTTACCTAAGAAGACCTCCCTTCTACGTTTTTT  
 TCTCCATGAAAGACAAGAAGATGTTGTGTAGCAAAATGGATAAGCAGAAAGAAATCTAA  
 AGTCTGTGTTAAATTTTCAGAGTAGCATGACTAAGGCAGGAGACAACAAAGTAAGAATATT  
 GAGAAGTAATCTGTGGCATCTCTCATCTTATGCTGTTTTTCTAACAAACACAAAGGA  
 GAATAAATTACATTTTCATGAACATTTTGTGTTTAACTTCAGATAAAGTAAATTTATGGC  
 CTTTTCACGATCTTGATTATTATGGAATCTGACTATTCCTTTTAAATGTTGAATGTT  
 CACTTTTACCATTAGATCTCAATTCATCTACTTTCTTAGAAGAGCCACTCATTTTATT  
 ATTCACCAAGTAATTTGTTAAATACCTAATATATGCTAATTTGTAAGCACTGGAGATAG  
 AGCAGTCTTCTGAAAGTTATATCTAGCAGTAAAGATATACAAATAAATTTAATAACCACA  
 TAAATTATACAAATGTGGTAAATCTATAGAGGGATATAAGCAAGGGGGCATGGGTTTGCT  
 AGAAGTTTCAGTTTGAATAATTAGGGCCGACTTCACTCAGAAGTTGTCTATTGGAACCTAAAG  
 ACTTGAAGATTGAGAGCAATGTGGATACCTGGGTGATAATTTCCAGATGACTGCAAGG  
 CCTGGGTAGGAACAAACCAAGTGTGAGCAGGAGGCTAATGAGGCCAAGTGAGTTTCAG  
 GGAAGTAGCAAGATAATGTTAGGGAAGAGGCTTAAAGGATCAACAAAGGGCTATTAC  
 AAAGGGATGGTAGGCCACGACAAAGGCTTTGCTTTTATTTGAATGAAATAGCCATTGAC  
 TGAGCATAAAGTGGCATGATTGATTCTGTTTTTAAAGGGATCACTTCGGCTACTGGGT  
 TAAATTAATATGTGGCTCAAGGATGGGAGGAGGAGAAATGACCTCTCAGGCTG  
 AGTCAAGCACCTGTGATATATACACTTCAGTACCATAGACCTCCCTGTGTGTAGACTA  
 ACCACAGTTTAACTTTTGTGTTTTCTTAGTGGTGTGATGTCCTTTTCCCTTCAGCAATGA  
 AAGATCCATGGAGGAAGGTCCATATCTAGTTTATATCTGAGTGTCTTGCATGGGGAA  
 TGTGCTCAGTAAACATTTGCTGAATGAAAGAGGAATAAATTTGCAAAATATCCCATTT  
 TTCCAAAGTTGAAACATGCTTTTTTGTATCGATTACAGGAACAAATAAATATTCATTAA  
 CTTTTTAAAGACATTTTTTAAATGTCTTTATGTGAGCAATTTATTACTAGTTTCAGAAA  
 TACTTTAAATGAGATTTAGATAAGTAGTAAATCAGCAATGTCAGTTTATTATATACG  
 TATGATACAGGATCATCCGATGTTGAATGATGAAGAGAAATTTGAACTGGAATATGTC  
 TCAAAATGACGGCTATAAAGAGGACACGAGAAATGTAAGATACCTGTATCTAAAGCAAGA  
 CCAAGTTTATGAGGCGTCTCAGAAATCAATGCTCAGATTATTTTTTTATTCTGTAGC  
 TGTCTTCTGGTGGAGTATAAAGCAAAATAGAACTATGACATTATTACTACTGAGATAA  
 AATTAAATCAGAAACTGTGTCCTCCCAAAATCATAGGAATGAAATTTAACCTTCAGGAT  
 GCGTTATTGGATAGTGTAAAGGCGCTGGATTCTGAGTCAAGAAAGATTCTCATTCCAAT  
 GGCACCTCTCAACTTTCCATCTGTGTGATCTTGACCTCTGAGCTTTGTCTATTTTTACA  
 TCACTATTTTGCTACAAAGCTGGACTTTATCATATTTCAGGACCTTGGACTTTCAGTGCAT  
 TTACCTTCGGATTACAGCTTAGAGGGAACTACTTCCCATCTGCAAGAGATACCAACTCA  
 ACAGGACAAACATTTACAGCTTGACCCAGTGTACATTTTCCATAAGCAGTCTTAGAATG  
 CATCTGTGTTATTTATCTAGGCATTATATGCTTAAATGTCGATCCATATACATGAGGGC  
 TATGATTCTGCTCTCTCTGTTTACCCTCCCATCCAAATTTCCAGATGTTTATAATCATTTA  
 TTGATTATGCTGTATATATGATTCTTTAATTTTCAGTACCTTGTCTGTTTATTTAATGTA  
 CTTCACTTCCATCATTTCAGTTGGATTACTCTGAAACCTCTTAACTGGCATCTTTTTTT  
 TTTTTTTTTTTTTTTGAGACGGAGCTTGTCTGTGTGGCCAGGCGGGAGTGCAGTGGCG  
 GGATCTCGGCTCACTGCAAGCTTCGCGCTCCCGGGTTCAAGCCATTCTCTGCTCAGCCT  
 CCGGAGTAGCTGGGACTACAGGCGCGCCGATCACGCGCGGCTAATTTTTTTGATTTTT  
 AGTAGAGACAGGTTTCAACGCTGTAGCCAGGATGGTCTCGATCTCTGACCTCGTGATC  
 CACCAGCTCTGGCTCCCAAGTGTCTGGGATTACAGCGTGAGCCACCGCGCCGCGCCAA  
 CTGGCATTTCTTACTCTGCTATGCTGCTTCTCTCCCATCATGCCACTGTCCACACAGT  
 GATTGTTCTAAATGCAAAATGCCCCCTGCTTGAAGCTGGCCCCCTGCTCCACATTGCCCTT  
 TAATTTTAAAGTTCAAACTCCCCATTAGGCTCTCTGATGAGCCCTTAACTCCAGTCTTC  
 TCAGGATTCTTCCATCTCTGCTTGAATGATACCCCAATGATTTTCCGAGATTCATGGT  
 AATGGACTGTGACTCTCCCCCAATCCAACCTTGATATAAAGCTCTCTCCACCAAGCAG  
 TCCCATTAAGTCAAAATGACTTTGACCCCCAGTCTGGGTTGCTGGGTGACTTTCAC  
 TCATTTTTCAGAAATCAGCTCAAAAGTCTTATTTCTTAACTCATCTCTGGCACATCAG  
 AAAGTTGACCTAGAGTCTCTCATATTTCTGAACCTTACCTTACACATGTGCCCATCAT  
 AGCATTTAATTCAGTTTAAACCCGCACTAGATATATAACTATAAATACAAAATTTAA  
 TTAAGAAGAGGATCAACGTTTCAGTGATCACTGTAAGCTAATTTATACCTATATCCATT  
 CCTATCTAGGGCTGGCATGCAATAGTAAAGGCCCAATAAATAGATTGTGGCATATATG  
 AATGAGAAAATGAATATAGATCACCTTTCCAGCTATTAAGAAGAAGAAAAGAGATAGGA  
 ACATTTACTCTAACTCTCCCATTTTTTTTCAGGAATCACACTGTCTCACAATGACC  
 ACAATCAACACCCACCTCCGGGAACTCTCCCTAAATCCCTTATGTAAGGCCATTGAC  
 ATGATCTGATGGGTGCTTTGCTCTGTTTCATGGCCCTTCTGGAATATGCCCTAGTC  
 AACTACATCTCTTTGGGAGGGGGCCCAAGCCCAAGAAAGCAGCTGAGAAGGCTGCC  
 AGTGCCACATGAGAAGATGCGCTGGATGTCAACAAGGTAATTCAGAGGGCAGGCC  
 TCTTGTCTTAACTCATGGAAGATGCTAGCAATACAGGTTGATGACCTTGTGAGC  
 AATAGGAAACCAAGCCAGGGAAGAGAAATGGTAATTTTCCCAAGCACACAAACAA  
 TTAATGATGGAAGCACAAATAGGACCAAACTCATGACACAGATTGGATGCCCTTCC  
 ATTAACAGAGGAGTGACCAACATGAGAAGACCTTATGATGCTTCTCACATATTGAG  
 TATAGTACCTATAATTTATGCTTCTTAAATTTATCACTAATTTATTTAAAGAG  
 GAAATTTAATGTTGCTTCAGTCAGTATTACTTGTGCAATAGCTTTACAA

Fig 1b

ACATATTACTCTAACTCTCCCATTTTTTTTCAGGAATCACAACCTGTCTCACAATGACC  
 ACAATCAACACCCACCTCCGGGAACTCTCCCTAAAAATCCCTATGTGAAGGCCATTGAC  
 ATGTACCTGTATGGGGTCTTTGTCTTCGTTTTCATGGCCCTTCTGGAATATGCCCTAGTC  
 AACTACATCTCTTTGGGAGGGGGCCCCAACGCCAAAAGAAAGCAGCTGAGAAGGCTGCC  
 AGTGCCCAACCAATGAGAAGATGCCCTGGATGTCACAAAGTAAATTCAGAGGGCAAGCCC  
 22. TCCTTGCTTCTTAAACTCTAGGAGAATGTAGCAATACAGGTTGATGACCTTGTGAGC  
 82. AAATGAGGAACCAAGCCAGGGAAGAGATGGGTAAATTTCCCCAAAGCACACAAACAA  
 142. TTAATGATGGAAGCACAAATAGGGACCAACTCATGACACAAGATTGGATGCCCTTTCC  
 202. ATTAACAAGAGGAGTGACCAACATGAGAAGACCTTTATGATGCTTCTCACATATTGAG  
 262. TATAGTACCTATAATTATTTATGCAITCTTTAAATTTATCAACTAATTTATTTAAAAAG  
 322. GAAATTTAATGTTGCTTCAGTCAGTATTACTTGTCTGGACAATAGCTTTACAAATATATAA  
 382. GTAAACAATTTAAAAAATATATCAATTAAGTCTAATTTATACCTAGATGAAGTCTCTGGC  
 442. CTTGAAATCTGCAATCTCTTTGACTTAAAGGAAAGATAAGCAAGTATACAGATGTGTAC  
 502. AGACCCACCAAGCAACAACTGAGACTTTTTCTTTGAAGCGATCAGAAGACATAAGAAG  
 562. AGTAAGGGGGAAAAGACAAATAATTTTCTCCTTTAAATGACAATGCCCTTGACATATACC  
 622. ACTTATCATTTGCAGCATTTGACTATATGAGGCCACCATCTTGTCTTAAGTCAGACATGGTC  
 682. AAAGATTCCCAACAACTATAAACCTGTATTATTTGTTAAGTCAGTATTTTCAATCATG  
 742. GGTGCCAATGGAATGACCTGAACAGATTAAAAAAAATACTGAAGCCTTGGTCCCTCCCC  
 802. CAGAAAATCTGATTTGATTTTCTTGGAGAAAGCATGGGCAACCAATTTTAAAAAGTTCC  
 862. CCAAGTTATTCTAATCTGCATCCAAATTTGAGAATCACCCTTAAAGGTTTGTAGGTA  
 922. GTCCAGTAGAGCCAGCTCTCTTACTCTCAATATTAACCTTCACTGGCTTCTGTAT  
 982. AGCACTTGCTGCCTAACAGAACTATGATATGATCTTCCAGAGGCCACATTTAGAC  
 1042. CTTTAGCAATAGCCAAAGAAAAGTCTTATTTTCATATCTGCCCTAAAATAGTCTTATGAA  
 1102. CTAGTGTCTAATATTGGACCAATAAATAGTTTATCATGATGGGCACTAGGAAAACATTAAT  
 1162. CAACTCTCTTAAACCAATTTGGAATTTAATTTAACAAGCTGATTTATTTCTCTGATG  
 1222. TTTTGATATTCTTAACTTCAAAATTAATTTCAAGGATAGCAAGTTATAAATAATCTTGCC  
 1282. ATTGAGTTGATTTGGAGCAACCATATGGAAGCTTCAACTCAGTAATGTTTTCACAT  
 1342. ATCCATAGGCTCTAATTTGGTTTATGAGCTCTCTCTAATCCAGGCACTCTCTGCTTAG  
 1402. AAGATGATATTTGCAACCAATGCAAAATTTAAGTATGGCCCTTGGAAACCAAAAACCGTA  
 1462. AGGCTCTAGGAAATTTGCAAAATCTTCCACACCCCAAGGTTTACTTATTGGAAATCTGC  
 1522. CAATAGATTCCAATTTACCATTTATGTTCTCTCAGGCTGTCAAAATGAAGAGTATTTTCT  
 1582. AAACCTATTTTCTGATTTTCTGACATAGTAGTCTTAGAGAGAGAGAGAAATAAATAA  
 1642. TAAATAAACCAACAAATAATAGCTATCTCATAGGACTTCAAAATGAGTCTAATCAATGTC  
 1702. ATAATGAAGGCTGACTTAATAAGATAACATCTGATCTCTCACTTTCACTCAACCTATT  
 1762. TTTTATACCAATTTGCAATTTAAATGATAAAGTTAAGACCTAGAGGGAGGAGGTTTATAC  
 1822. AAACCTGAACCTGCAGAGCTTCAAGATAGTTCTAAAACGGAAATAGAGAAAAGTTCACTAGG  
 1882. CAGTTACCCCATAGTGGTGAAGAGCTATCTCTTGGTTGAAGGTAGACCTACCATTTCA  
 1942. AGTATGGAATATGAGAGAACAAAAGATACAGAGATTATTAGTATTTTCTAGTGGT  
 2002. TCTCAAAACAGGGTCAATTTCTCTCCAGAGGACATTAGGCATTGTCTGGAGACATCTT  
 2062. TCATTTTAAAACTATAGGATGAGGTTTACTACTGGTATCTAGTGTCTAGAGGCCAGGT  
 2122. TGCTGTACACATTTGGCAGTGTGCGAGGATAGCTTCCCTGTAACCTTCTGTTTCAACA  
 2182. TGAGAAATCTAGGACTGAAAAATAATATCTCTTGTGCTAATAATTTCTTAGCAAGT  
 2242. TGTCTCTCCATTTCTTTGAGCAGCATGTGAAGAAATCAAGACATCATGATCATGTTT  
 2302. AGAACCAATGTTCTGAACAAATATTTAGAGTCAAGTTCAATATTTACCTTCCCATTTAA  
 2362. CAACTGGGTGACAGTAGACAAAATTTAGCCTCTCTGGCCCTTTAGTTTCTCATCTGT  
 2422. GAAACAGGGTTAATCTCTCTTATAAAGTTACTCATGAACATAATGTGATAATGTGCA  
 2482. AAGAGGGTAATATAGTCTCAATACATGTTAAGCACTCAGGAAGTGATAATATTAATAA  
 2542. AATATTAAATAATAAAGTGGTAGGCATTATTACTTTTAAAAATAAATTTGAGTCC  
 2602. CATACTTATTGGTGATGCTAGCGTGTGTCTATATGCTCTACCATGTGGAATGCAAGTA  
 2662. GCATCCCTAAAGCAGTGTATTGTTTAGACAGGAATGTTTATGTTAGAAATTTTAAAGTC  
 2722. AAATCAAGAAATCATTTTCAAGGGTATTAGCTATAAATCTATAGCAATCAACTGC  
 2782. ATCAAACTCTATTAATTAATTTCTTAGAGCAGAAAACCTACAAACCATTTTGATTAGATTA  
 2842. AACCAAAATCTGACCATCAAGCTTTCCAGACTCCCACTATTCTTTGTTCAACCCAGCAT  
 2902. GAAAGGTTAGAGGAGGTCACATTTGCTCTGATTTATGCGTGTCTGCTTTGTGTGAGCA  
 2962. TCAATGAATAATACCAACCCATTTCTTATCAAGAAATGTCCTCTTTTCAACTGTAATCT  
 3022. ATAGTTATTTTTTTTTTCCAAGATCAAGCACTTATAATCAGCTAAGCATTTGTTGGCTGCT  
 3082. CTGGGAAAATCAGGAGTGGGATTAAATTTTTTACCTTCTCTTGGTATTTCTTAAAGATG  
 3142. GAAGGAGTGTGTGAGATTATCTAAAGTGAATTTTGAACCTGTGCTCTGCTCCTGAGGTGAG  
 3202. GAGGGTTGAAAACCGGAGGCTGCCAGGTGGGAGCCAGTGTGATTCAGCAGATGCTA  
 3262. CCGCATTTTTTTCAGCTCTTTTGGTATTAGGCTCTCTTAAATGTTTGTTTTAAAGT  
 3322. GAATTTGCAGCTCTTAAATAATTTCCGCTCTGTCTTTCACTGACGTAAAAGCCTATGT  
 3382. GCCTAAGAAGGTTGAATAGCCAGGTCATAGCAAGCTTTTAAAGTTCTGATATGGA  
 3442. TTTTCTATCTCTCCAGTGTACATAAAGTGTGCTGGCAAAAAATAAATAAATAA  
 3502. AAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA  
 3562. TTCCAACCTCAGTTGATTTACCTGAAAAATCCAGCTACCTGCACATGAAAGTTTATAAG  
 3622. CAAGTGTCACTTTACCGTGTCTTCAATGTGCCAAGTTAAGTAGTAAAGTACTAAGATT  
 3682. TAAAAATAAACTTTAAACCAACATGAGGCTACTAGATATTTTTTCCCAAGTGTCTTTG  
 3742. GAGTAGTTTAAACATGATCTTTATCTAATGCTAGGAGAGTCTTTCTATTTTCCCTTGC  
 3802. ATTCTTCTAGTTTGTCTTAA  
 3862. TGGAAATTTGCTTTGATTGATTTCAACCACTGTCTTGGCCGCTAGCTTCAAGTTTCAACT  
 3922. CCCCTTACTTCTCTTTAAGCTTTATATTGTTTCTCTGTTTATTTTTCTGCCAAATACTA  
 3982. AGAACACGCATATTGAGAATCAAGTTATTTCTGCTTTTACCACCGTGGTATTGACAC  
 4042. ATATCAAAATGCACTGACGCAACAGTTTCTAATCAAGCTGACCAACAGAGATCTCCA  
 4102. TTTAAGGGTGTCTTTATCTGCATTATGAGGCTGTGTGACTCTTTGTGTGTCTTTATC  
 4162. CATTTGTCCTTAACTCTTTCTAATGTAAGGCCACTAAGATATTTCAATCAATTTATTTCT  
 4222. ACTGCTTGGAAATTTGATGTTTAAAAACAAACATTTTTTAAAAACTTACTCAAATCCC  
 4282. ATCTCTGTTAGCCTTTATCTAAGGATTTCTAAGGCCAGTAGCATTTATCTATATCCA  
 4342. ATTTACTTTAAACCATCAGGCTAATCTAGTAGCATCAATCGACTTAATTAGCAATCTC  
 4402. TACTTTGTCTTCCAGATTTTCTAABAGATTTAAACAAATGGGACCCATATCGATCC  
 TTTGGGACCTTCTGGAABCCCTCTCCCACTAGAGCGGACTACCAATTACGATTTCTCT  
 CTGATACCGTTAGATAGCCAGTTTTTAATCCATTTATTGTTTCTGTTGGGGCCAAAT

Fig 2

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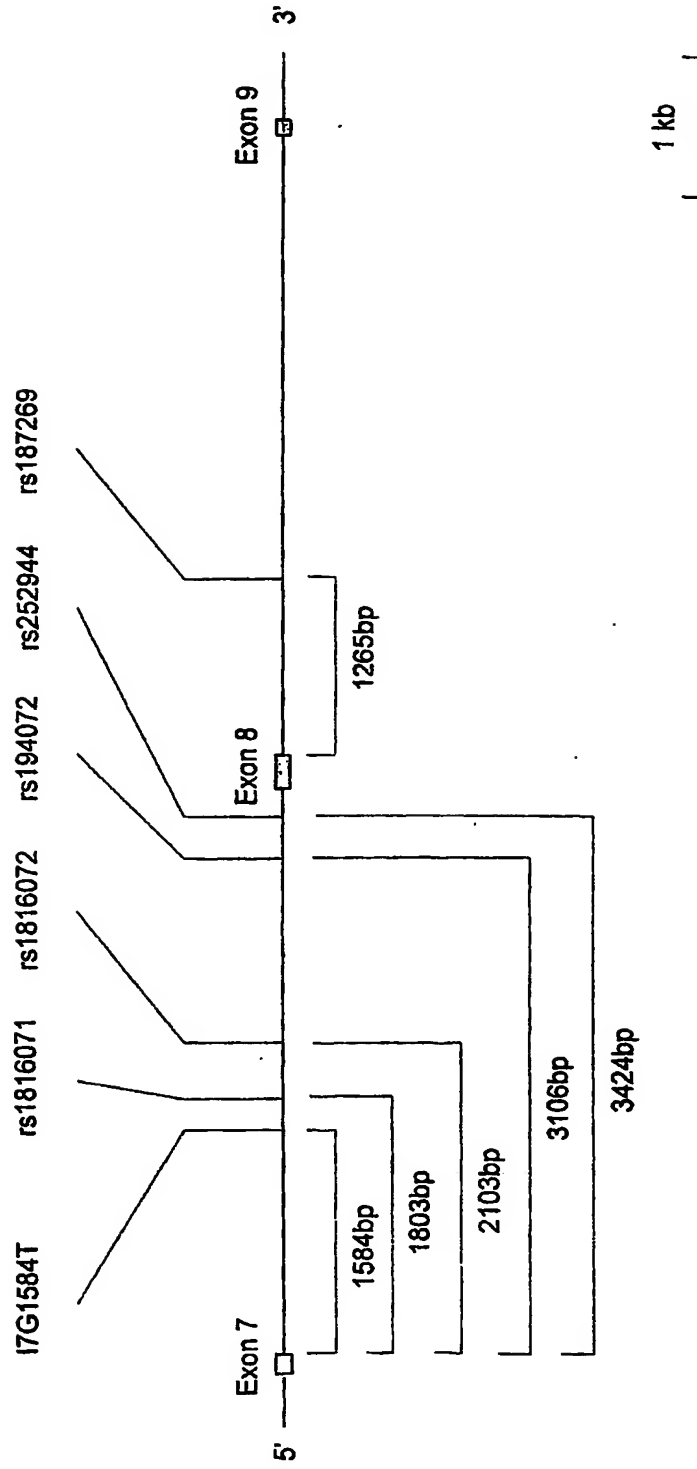


Fig 3

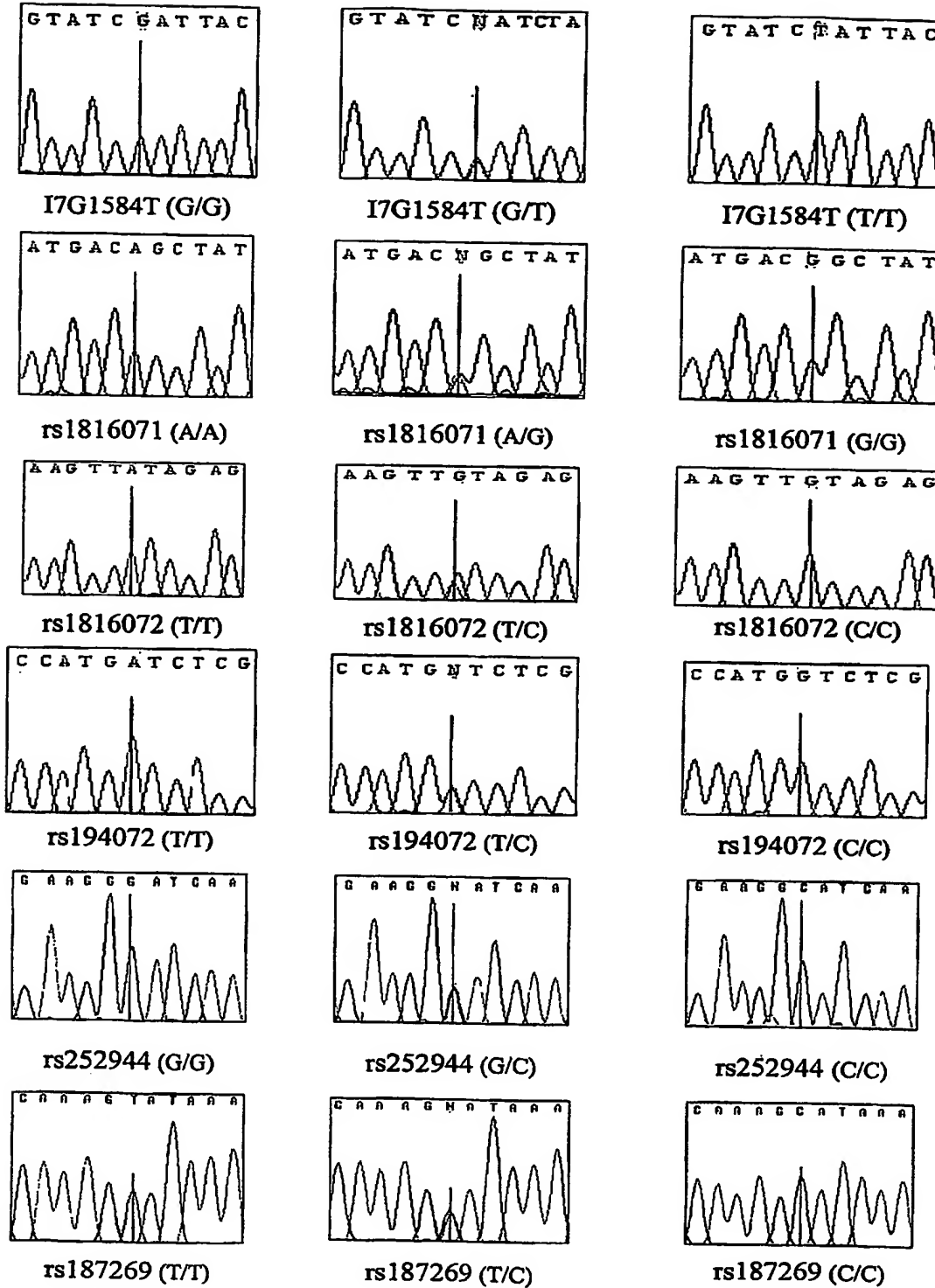


Fig 4



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